ELECTROPHYSIOLOGICAL STUDIES OF ARTHROPOD CHEMORECEPTION. III. CHEMORECEPTORS OF TERRESTRIAL AND FRESH-WATER ARTHROPODS[1]

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While an extensive literature documents the role of chemoreceptors in the behavior of invertebrates (Hodgson, 1955), the small size of chemoreceptor cells is a major handicap in any attempt to study their functions using conventional electrophysiological procedures (Chapman and Craig, 1953; Roys, 1954). Barber (1956) recorded afferent impulses from neurons which supply the gnathobase chemoreceptors of *Limulus* and noted an increase in nerve activity when aqueous extracts of marine bivalves were applied to the gnathobase. Use of microelectrodes enabled Schneider (1957) to record afferent impulses from groups of antennal chemoreceptors in male silkmoths (*Bombyx*) during stimulation with extracts of the scent glands from female moths. Possible synaptic effects between receptor cells and nerves supplying them, or the unpredictable numbers of cells represented in most recordings, make it difficult, however, to interpret the results in terms of single unit activity of the actual chemoreceptor cells.

A relatively simpler technique is that of recording the afferent impulses from primary chemoreceptor cells through the same fluid which is applied as a stimulus (Hodgson, Lettvin and Roeder, 1955). This method has thus far been applied only in studying contact chemoreceptors of two animals: labellar chemoreceptors of the blowfly *Phormia* (Hodgson and Roeder, 1956; Wolbarsht, 1957) and tarsal chemoreceptors of the butterfly *Vanessa* (Morita *et al.*, 1957). The conclusions from studies of these two preparations point to a number of unexpected properties of primary chemoreceptor cells.

With both *Phormia* and *Vanessa*, it was found that different chemoreceptor cells were specialized to respond, not to the different modalities of stimuli generally held to be effective for contact chemoreceptors of vertebrates (*e.g.* Beidler, 1952), but either to sugars or to various non-sugars, with the presence of a water-specific receptor also strongly indicated in *Vanessa* (Morita *et al.*, 1957). Seemingly at variance with the usual concept of single specificities of receptor cells (Granit, 1955), a single primary receptor cell of *Phormia* may respond to chemical, tactile, and thermal stimuli within normal physiological ranges (Hodgson and Roeder, 1956). Unfortunately, information on this point is not available for *Vanessa*.

In view of these unexpected results, and the lack of any comparable electrophysiological data on primary chemoreceptors of other invertebrates, it seemed desirable that the method of recording through fluid-filled, externally applied electrodes

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should be tried on chemoreceptors of a wider variety of animals, in order to determine how generally the characteristics found in *Phormia* and *Vanessa* receptors may apply to the functions of other primary chemoreceptor cells. For technical reasons, this method is best adapted to recording from chemoreceptors in arthropods (Hodgson, Lettvin and Roeder, 1955). The object of the present paper is to report the results of tests conducted using this method upon the chemoreceptors of some terrestrial and fresh-water arthropods. In each case where the method could be successfully applied, answers to the following questions were sought: (1) Does the same receptor cell respond to chemical, tactile, and thermal stimuli within normal physiological ranges? (2) What modalities of chemical stimuli excite the individual primary chemoreceptor cells? (3) Does the relationship between the reaction of the animal to chemicals and the range of sensitivity of its chemoreceptors indicate a peripheral discrimination mechanism, such as found in *Phormia*?

Methods

Thirty-seven species, representing the major classes of arthropods and eight orders of insects, were tested. These species are arranged according to taxonomic status below. All specimens were collected in the field and tested within 12 hours after capture. The animals were allowed to drink water to repletion, but no attempt was made to control their diet prior to testing. At least three individuals, usually more, belonging to each species were studied.

The technique of recording action potentials from chemoreceptors using externally applied, fluid-filled electrodes has been described in detail elsewhere (Hodgson, Lettvin and Roeder, 1955; Hodgson and Roeder, 1956). This technique was used with only such minor modifications as were necessary to manipulate the variety of receptor-bearing appendages tested. All experiments were tape recorded and photographs made from the tape recordings, beginning one-half second after the stimulus was applied, thus avoiding the base-line fluctuations which commonly accompany the stimulus artifact.

The species tested were as follows, with each group and each species yielding potentials from chemoreceptors designated by an asterisk. (Except as otherwise noted, identifications were checked through the courtesy of Dr. R. E. Blackwelder of the U. S. National Museum.) *Class:* Crustacea*—Cambarus bartonii sciotensis* (Det. H. H. Hobbs, Jr.); *Class: Arachnida*—Latrodectus mactans (black widow spider), Theridion tepidariorum (house spider); *Class: Diplopoda**—Pseudotremis sp. (Det. H. F. Loomis), Pseudopolydesmus serratus* (Det. M. Walton); *Class: Insecta; Order: Odonata*—Aeschna constricta, Libellula pulchella, Progomphus obscurus; *Order: Orthoptera**—Acheta assimilis (common field cricket), Ceuthophilus gracilipes* (cave cricket), Crytocercus punctulatus (wood-eating roach) (Det. L. R. Cleveland), Hadenoecus putaneus* (cave cricket), Scudderia furcata (katydid); *Order: Hemiptera*—Arilus cristatus, Oncopeltus fasciatus (large milkweed bug); *Order: Coleoptera*—Cicindela sexguttata (six-spotted tiger beetle), Dineutes americanus (whirligig beetle), Dytiscus fasciventris (large diving beetle), Laccophilus maculosus (common pond beetle), Nicrophorus tomentosus (carrion-beetle), Phymatodes dimidiatus (longhorn beetle), Saperda candida (apple tree borer), Silpha americana (carrion beetle), Tropisternus lateralis (keeled water beetle); *Order: Megaloptera*—Carydalus cornutus (dobsonfly); *Order: Neurop-*

tera—Chrysopa sp. (golden eyed lacewing); *Order: Diptera**—Amoebaleria defessa* (cave fly) (Det. C. H. Curran), Tipula trivittata (crane fly); *Order: Lepidoptera**—Atlides halesus (purple hairstreak); Epargyreus clarus* (silver spotted skipper), Limenitis arthemis astyanax* (red spotted purple), Papilio marcellus* (zebra swallowtail), Papilio philenor* (pipe vine swallowtail), Protoparce quinquemaculata (five-spotted hawk moth), Speyeria cybele* (great spangled britillary), Tropaea luna (luna moth), Vanessa atalanta* (red admiral).

The chemicals tested were sodium chloride, sucrose, d-levulose, glycine, DL glutamic acid, citric acid, oil of citronella and oil of wintergreen. Sodium chloride was tested as a 0.25 molar aqueous solution. Oils of citronella and wintergreen were tested by bringing swabs soaked in these chemicals to within an inch of the sensory structure. Although quantitative control of stimulus concentration was not obtained by this method, the results obtained with these two oils were quite reproducible. All of the other chemicals were mixed with sodium chloride so that the final test solution was an unbuffered aqueous solution containing 0.1 molar NaCl and a 0.25 molar concentration of the test chemical. Results were compared with activity recorded when 0.1 molar NaCl was applied alone.

Temperatures were measured with a thermistor implanted just under the cuticle near the receptor being studied. The temperature was changed by bringing a warm glass rod or small ice-pack near the preparation. Spike potentials from mechanoreceptors were recorded by bending sensilla or whole appendages with needles. Certain departures from the usual tests are described at appropriate points below.

Results

All of the preparations yielded numerous spike potentials originating from tactile receptors, thus providing assurance that the preparations were alive when studied. In only five orders of the arthropods tested, however, was it possible to obtain unequivocal recordings from chemoreceptors. These five groups are designated by asterisks above. The several factors believed to be responsible for failure to record action potentials in all of the tested species are considered in the discussion, and a complete description of the results will be presented only for those forms in which chemoreceptors could be studied using fluid-filled electrodes.

1. DECAPODA *Cambarus bartonii sciotensis* (16 individuals)

This large crayfish proved to be an exceptionally interesting experimental animal. Recordings could be made with the usual 0.1 molar NaCl conducting solution in the electrode, or else by using distilled water or pond water as a solvent for the chemicals. Although the results showed few differences whichever solvent was used, all of the tests were run with chemicals dissolved in distilled water, thus avoiding any possible complications of the sodium chloride.

The antennae and the lateral branches of the antennules were alike in yielding only records of mechanoreceptors at low amplitudes (30 μV). From the entire medial branch of the antennule, however, it was possible to record a variety of spike potentials ranging in amplitude from 30 μV to 500 μV. The large-amplitude spikes (200 μV to 500 μV) were recorded only when the antennule was bent. Consequently, the cells giving rise to these potentials, which are relatively few in this

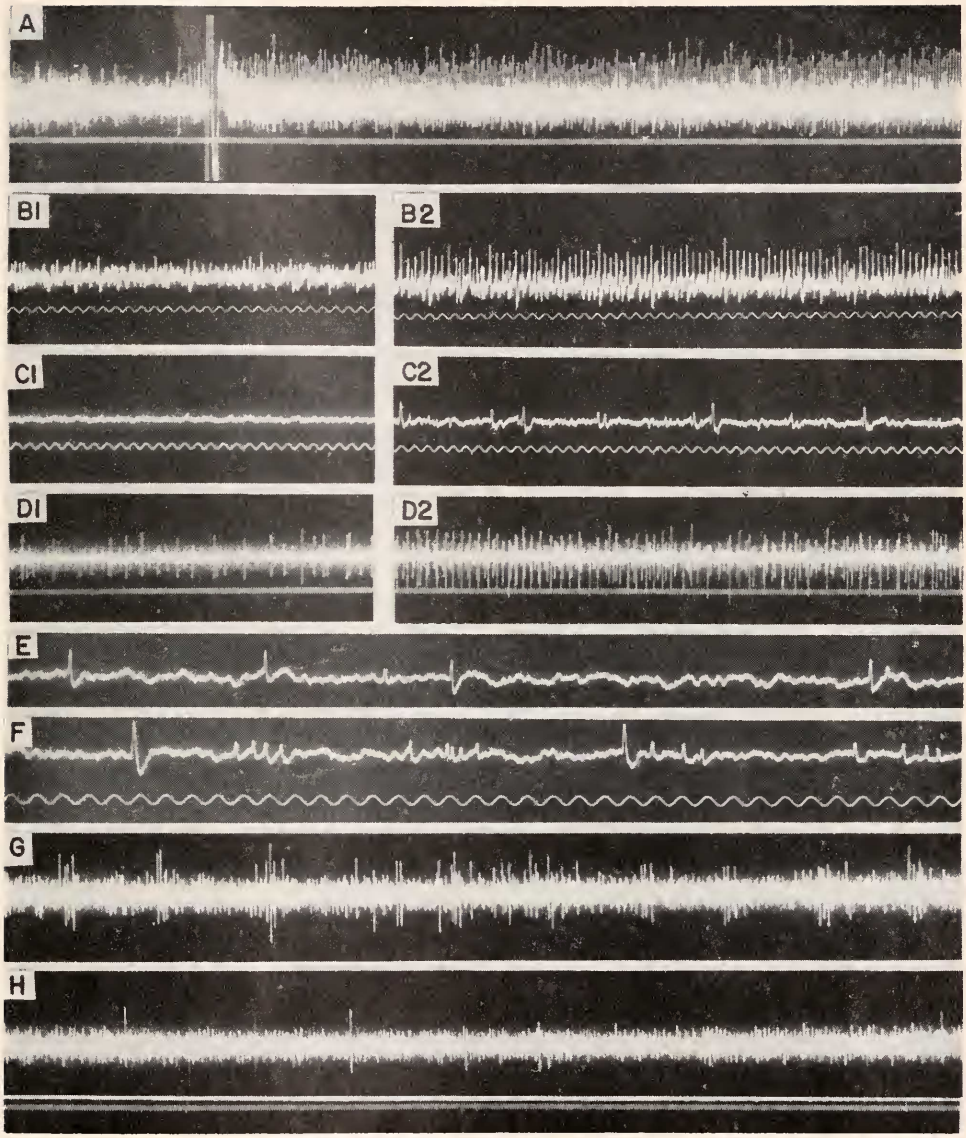

FIGURE 1. Typical spike potentials from arthropod chemoreceptors. *A*, response of medial branch of *Cambarus* antennule to glutamic acid; *B1*, single sensillum on *Cambarus* walking leg, tested with distilled water; *B2*, same as *B1*, except glycine test solution; *C1*, *Pseudopolydesmus* tarsus, NaCl control; *C2*, same as *C1*, except sucrose test solution; *D1*, spontaneous activity, *Hadenoecus* tibia; *D2*, same as *D1*, except exposed to citronella vapor; *E*, single tarsal sensillum of *Epargyreus*, control NaCl solution; *F*, same as *E*, except sucrose test solution; *G*, antenna of *Amocbaleria*, distilled water in electrode; *H*, same as *G*, except exposure of antenna to oil of citronella vapor. Time bases for all records, 100 cycles per second. Consult text for additional details.

branch of the antennule, are mechanoreceptors. The majority of the spikes have amplitudes of 30 μV to 50 μV. These respond to the application of glycine and glutamic acid, of the test series of chemicals used. Because a number of different amplitudes of spikes were recorded even with the smallest practicable areas of electrode contact, it was not possible to determine whether identical cells were responding to both chemical and tactile stimuli. Record A of Figure 1 is taken from an experiment in which a test solution of glutamic acid was allowed to flow around the medial branch of an antennule without changing electrode contact. Activity recorded when the antennule is in distilled water (on the left of the large stimulus artifact) is negligible, but many small-amplitude spike potentials follow the introduction of the glutamic acid. The frequency of firing during chemical stimulation was not influenced by temperature changes within the range tested—five degrees (C.) above or below the room temperature of 25 degrees.

Chemoreceptors were also found on the first two pairs of walking legs. The chemoreceptors were located on the chelae and, to a lesser extent, elsewhere on the protopodites of those legs. The external chemosensory structures can be recognized in *C. bartonii* as tufts of setae, numbering ten to twenty setae per tuft, each such tuft arising from a circular depression in the cuticle. Contact of the electrodes with other parts of the cuticle failed to terminate the open circuit condition between the indifferent electrode inside and the recording electrode outside the cuticle. The best records were obtained after the claw had been allowed to dry at room temperature for thirty minutes following its removal. This prevented short circuits between the recording and indifferent electrodes. By teasing apart the hairs of a single tuft, the tip of an electrode could then be positioned over a single sensory hair. In this way the firing of a single chemoreceptor cell could be studied. The spike potentials recorded from different sensilla ranged from 30 to 60 μV in amplitude. It was found that these receptors resemble chemoreceptors on the antennule in being insensitive to test chemicals other than amino acids of the series used. (Records B1 and B2 of Figure 1 illustrate typical results during applications of a control NaCl solution, and the test mixture of NaCl and glycine, respectively.) The chemoreceptors on the first two walking legs were never observed to respond to mechanical movement of the sensory hairs during recordings. The small size of the hairs (about 20 microns in length) and their position surrounded by rigid cuticle would appear to render their usefulness as tactile receptors unlikely. The insensitivity of these receptors to temperature changes within the range tested resembles that of the receptors on the antennule. Impulses from chemoreceptors were not detected from the chelipeds, third maxillae, or elsewhere on the body of the crayfish using the present method.

Behavioral experiments were run to check the possibility of a peripheral discrimination for amino acids. Ablations of antennae, antennules, or the first two pairs of walking legs, and combinations of these operations, were performed on thirty crayfish. The results were difficult to interpret in many cases because of abnormal behavior of operated animals. It was easy to demonstate, however, that the animals can locate food using the first two pairs of walking legs, even when antennae, antennules, and maxillipeds are removed. Activity resembles that during normal feeding and can be initiated by injecting 0.25 molar solutions of glycine and glutamic acid into the water, while even intact animals fail to give clear-cut responses to the other test solutions. Thus there seems to be a clear correlation between the elec-

trophysiological data and the behavioral results. Attempts to determine by behavioral tests whether the antennae and lateral branches of the antennules bear chemoreceptors yielded results which could not be unequivocally interpreted. Doflein (1910), on the basis of behavioral tests, has reported that the antennules of decapods contained chemoreceptors, and Luther (1930), using similar methods, reported chemoreceptors on mouth parts, walking legs, and pincers of brachyurans.

2. DIPLOPODA *Pseudotremis* sp. (4 individuals); *Pseudopolydesmus serratus* (4 individuals)

In both *Pseudotremis* and *Pseudopolydesmus* many action potentials could be recorded from the tips of the antennae and from the tips of the legs when an electrode filled with 0.1 molar NaCl was applied to those parts. In *Pseudotremis*, the smaller species, the action potentials were never more than 40 μV in amplitude, and all clearly responded to mechanical bending of the antenna or leg. In *Pseudopolydesmus* the largest spikes from the antenna were about 60 μV in amplitude, and those from the tarsus were about 80 μV. All of the larger spikes increased in frequency during bending of the appendages being tested, and it was therefore assumed that these spikes represented the afferent impulses from mechanoreceptors. Spike potentials of smaller amplitude (30–50 μV) from tarsi of *Pseudopolydesmus* occurred with increased frequency when the tarsi were bent, or sugars applied. (See Fig. 1C.) They did not change during application of other test solutions or during temperature changes within five degrees (C.) of the room temperature of 25 degrees. No significant changes in the frequency or pattern of impulses were noted in recordings from the antennae of the two species when chemical stimuli were applied.

The small trichoid sensilla which probably enclose the actual chemosensory cells on the tarsi of *Pseudopolydesmus* are too closely spaced to make possible a restriction of the area of electrode contact to a single sensillum. Attempts to record activity using electrodes filled with distilled water were likewise unsuccessful. In view of the smaller size of the mechanoreceptor spikes recorded from *Pseudotremis*, and the generally smaller size of action potentials from chemoreceptors as compared with mechanoreceptors, it would hardly be expected that chemoreceptor spikes from *Pseudotremis* would be detectable above the inherent "noise level" of the apparatus. Behavioral test showed that sucrose or levulose, placed in contact with the tarsi, initiated feeding responses even after the antennae were removed. Tarsal contact with citric acid caused the animals to move away from the test solution, but this was the only test solution, other than the sugars, which elicited a behavioral response. With the exception of citric acid, receptors for which could not be detected electrophysiologically, the behavioral and electrophysiological results suggest the existence of a peripheral discrimination mechanism.

3. ORTHOPTERA *Ceuthophilus gracilipes* (7 individuals); *Hadenoecus pubescens* (3 individuals)

The orthopterans tested showed considerable variation, some of which appears to be related to habitat. *Cryptocercus*, a wood-eating roach, was completely refractory to the recording method, except for a few mechanoreceptors in the antennae and palpi. A larger number of tactile receptors were recorded from the antennae and

palpi of the katydid, *Scudderia*, and the field cricket, *Acheta*. Best results, however, were obtained with the cave crickets *Ceuthophilus* and *Hadenoecus*, which have antennae elongated to many times the length of the body and also have unusually long legs and palpi. The data support the generally expressed assumption that these anatomical modifications are associated with hypertrophy of tactile and chemical senses which would presumably be of selective value in dark subterranean environments.

In tests of seven adult specimens of *Ceuthophilus* and three of *Hadenoecus*, the antennae were found to contain spontaneously active and quick-adapting mechanoreceptors (spike amplitudes 50–80 μV) along with spontaneously active, relatively non-adapting chemoreceptors (spike amplitude 20–40 μV). The latter were seen in one antennal preparation of *Ceuthophilus* and all three preparations of *Hadenoecus*. The frequency of the small spikes did not change during application of any of the test chemicals in solution, or during temperature changes between 20 and 30 degrees C., but did increase when swabs soaked in citronella or wintergreen were brought near the region of the antenna in contact with the electrode. Essentially similar results were obtained from recordings of the receptor activity in both the maxillary and labial palpi and the tarsi of *Ceuthophilus* and *Hadenoecus*. In addition, small spikes (30–50 μV) were recorded from the trochanter and tibia of the prothoracic and mesothoracic legs of *Hadenoecus*, in six out of eight preparations when the legs were exposed to vapors of wintergreen or citronella. Mechanical bending of sensilla on the trochanter and tibia also increased the frequency of these same spike potentials. Record D1 of Figure 1 shows the spontaneous activity of receptors in the tibia of a prothoracic leg of *Hadenoecus*, and record D2 shows the increase in frequency of spikes during application of citronella vapor. No effects of the test chemicals in solution could be detected in either *Ceuthophilus* or *Hadenoecus*, and chemoreceptor activity could not be recorded from the cerci, ovipositor, general body surface, or the larger spines on the legs of either species. *Ceuthophilus* did not give any clear-cut behavioral response to citronella or wintergreen in tests of the intact animals, but *Hadenoecus* gave intense avoidance reactions, moving quickly away from these stimuli. Removal of the antennae and palpi did not abolish this reaction in *Hadenoecus*, which always responded most strongly when stimuli were near the legs.

4. LEPIDOPTERA

Nine species of Lepidoptera were tested. Only a few impulses associated with tactile stimulation could be recorded from the antennae of any of these species, even when vapors were applied. In all six species of butterflies tested, records were obtained from the tarsal receptors (described by Minnich, 1921). Tests upon the tarsal sensilla of *Epargyreus* and *Limenitis* revealed that each sensillum had a few receptor systems functioning similarly to that in the labellar hairs of *Phormia*. (Compare the records E and F of Figure 1, taken from tests of a single tarsal sensillum of *Epargyreus*, and note that the small spike potentials predominate only in record F when sugar is present in the electrode.) The maximum number of receptors represented in recordings from single sensilla of these two species is four, and the minimum two. Variations within these limits were commonly encountered in comparisons of the records from several hairs, even on the same tarsus. The

variations characteristically occurred in the smaller spike potentials, but under the conditions of these tests all of the smaller spikes increased in frequency during stimulation with sugars, and the largest spike responded with increased frequencies during application of any of the non-sugar solutions. These receptors were not observed to respond to vapors of citronella or wintergreen.

With the other species of butterflies tested, there appeared to be as many as 12 different receptors associated with each tarsal sensillum and the records were too complex for analysis of the functions of any single receptor cells. Responses to tactile stimulation were obtained in tests with tarsal hairs of all the butterflies used; in those preparations involving only a few fibers it was clear that all fibers responded to bending of the tarsal hair, and probably this was the case with the many-fiber preparations also, but this could not be determined with certainty because of the complexity of the records. The frequency of impulses recorded during continuous stimulation of single sensory hairs of *Epargyreus* and *Limenitis* was increased by temperature rises of as little as 1.2 degrees C. These particular tarsal receptors, then, bear a greater resemblance to the labellar chemoreceptors of flies than do any of the other preparations (excluding the labellar chemoreceptors of *Amoebaleria*) encountered in this survey. Feeding responses (proboscis extensions) in butterflies are known to be elicited by sugars, with negative responses being elicited by other types of chemicals (Dethier, 1953; Minnich, 1921). A peripheral mechanism for discrimination of acceptable and unacceptable chemicals is thus indicated by both the behavioral and electrophysiological results with butterflies.

Tarsal chemoreceptors were not detected in any of the three species of moths. No impulses could be recorded from the trichoid sensilla described by Frings and Frings (1949) on the proboscis of lepidopterans. The characteristics of the records obtained from such tests indicated, however, that a short-circuit between the recording and indifferent electrodes, established through the fluids in the proboscis, probably accounted for the lack of any spike potentials detected through an active electrode near the tip of the proboscis.

5. DIPTERA *Amoebaleria defessa* (7 individuals); *Tipula trivittata* (3 individuals)

Studies on four genera of Diptera having been previously reported (Hodgson and Roeder, 1956), the present work was confined to two types in which the chemoreceptors might be expected to be of special interest. The helomyzid fly *Amoebaleria* was tested because of its occurrence in caves, a habitat often associated with hypertrophy of chemical or tactile senses (Hodgson, 1955), and the crane fly *Tipula* was tested because the branching structure of its antennae suggested that recordings might be made from one or a few antennal receptors in a single antennal branch. Only *Amoebaleria* yielded results of interest, however.

The labellar chemoreceptors and chemoreceptors within the tarsal hairs of *Amoebaleria* proved to function similarly to those in *Phormia*, in that they exhibited *L* and *S* spikes when stimulated by sugars or non-sugars, and showed comparable responses to tactile and temperature stimulation. Some data on olfactory receptors were obtained in recordings from the antennae of *Amoebaleria*. A typical result, obtained by placing a fluid-filled electrode on the antenna, is shown in record G of Figure 1. Distilled water is adequate in the electrode, and the results are essentially

the same whether contact is made with the distal tip of the antenna or the enlarged third segment near the base of the antenna. Ablation experiments show that most of the activity recorded originates in the third segment of the antenna in either case. The abundant spikes which seem to represent the basal level of receptor activity in the absence of externally applied stimulation are not affected by any of the test solutions applied, but are decreased in frequency by vapors of wintergreen, or citronella (see record 11 of Figure 1). This result was so contrary to anticipated findings that tests were run with benzene, toluene, and carbon tetrachloride vapors, all of which produced similar reversible decreases in amount of receptor activity. Unfortunately, so little is known of the natural history of this fly that it is impossible to say what might constitute the normal olfactory stimuli.

Tactile effects upon the antennal receptors were observed only when the surface of the antenna was prodded or bent in excess of any amount of stimulation which the antenna would encounter in flight. Blowing upon the antenna during a recording or varying the temperature from 20 to 28 degrees C. produced no discernible effect upon the frequency or pattern of the impulses recorded. Attempts to make similar antennal recordings using other species of flies have yielded only negative results.

Discussion

In view of the considerable differences in chemoreceptors which have already been reported from electrophysiological studies of mammals (Beidler, Fishman and Hardiman, 1955) it is not surprising that much greater differences should be found among members of such a heterogeneous group as the arthropods. It seems clear that sensitivities to tactile and temperature stimuli within the normal physiological range are not essential characteristics of primary chemoreceptor cells, even among the arthropods, because several exceptions to this situation were found as soon as tests were made of chemoreceptors other than those on the fly labellum. Yet it would probably be incorrect to regard the labellar receptors as primitive or unspecialized receptor cells. Their similarity to receptors in the tarsal sensilla of at least two of the butterflies tested suggests that a sensitivity of the same cell to more than one type of energy in the environment may have a high selective value in cases where only a relatively small number of receptors contact a substrate, many features of which are significant for the animal's behavior. This certainly would be the case with receptors on the tarsus or proboscis of a fly or butterfly, or on the tips of the tarsi of a millipede. The demonstrated multiple sensitivities of single receptor cells in those locations may, therefore, be one of the solutions which evolution has produced for the problem of obtaining a variety of information about the environment when only very small areas of the body are actually in contact with the environment. Whether the several types of stimuli all eventually affect the same excitatory process within a single receptor cell will have to be determined by further investigations. Cases of double specificities of receptors in vertebrates, such as the temperature-touch receptors of the rattlesnake facial pit (Bullock and Diecke, 1956), have been reported but it is very doubtful that more than one type of stimulation *normally* acts upon the same receptor units, and even if this were true these would have to be considered exceptions to the general rule of single specificities for single receptors (Granit, 1955).

Several correlations might be noted between receptor distribution or function

and the natural history of the particular animals concerned. Of the two cave crickets providing favorable receptor preparations, *Hadenoecus*, with the more extensively distributed chemoreceptors on the legs, is reported to be more strictly limited to caves than *Ceuthophilus* (Giovannoli, 1933). The selective advantage of highly developed chemical senses in a totally dark environment is obvious. The sensitivity of the chemoreceptors of *Cambarus* to amino acids is undoubtedly related to a diet of decaying meat, and the absence of any response of its receptors to sugars can be correlated with the lack of any behavioral response to sugars by this species. The results with butterflies likewise indicate the existence of a peripheral discrimination mechanism for the chemicals constituting the normal food in this case, sugars.

All of the spike potentials recorded from chemoreceptors were smaller in amplitude than the spikes from mechanoreceptors of the same animal, unless the same receptor cell responded to both types of stimuli. This is in accord with the usual assumption that chemoreceptor fibers are smaller than mechanoreceptor fibers (Dethier, 1953; Hodgson, 1955). The fact that many receptors in *Cambarus*, *Hadenoecus*, and *Amoebaleria* showed spontaneous activity supports another idea believed to be of some general applicability—that spontaneous activity is widespread among sensory cells, and that *any changes* in the frequency or pattern of the spontaneous activity (rather than the mere presence of impulses) may constitute the afferent "message" from the sense organs (Roeder, 1955). The antennal receptors of *Amoebaleria*, showing decreased numbers of impulses during administration of vapors, may illustrate a less common direction of change in spontaneous activity which serves as the afferent message.

The present experiments resolve a discrepancy between the earlier work on the labellar chemoreceptors of the blowfly (Hodgson, Lettvin and Roeder, 1955) and the results obtained by Morita *et al.* (1957, and personal communication) using the butterfly, *Vanessa*. The polarity of the spike potentials recorded from *Phormia* was previously reported as negative, using the present recording method, but positive under similar conditions in *Vanessa*. All the spike potentials recorded from chemoreceptors in the present study resulted from an increase in positivity at the distal tip of the sensory hairs (position of the recording electrode) relative to the base of the same hairs (position of the indifferent electrode), and the contrary polarity reported in *Phormia* was subsequently traced to an error in instrumentation. A precise explanation for the positive spike potentials obtained by this method cannot be given at the present time, but might possibly be explained by generation of the main negative spike potential at the cell body region of the receptor, which would leave the actual chemosensory area with a relatively positive charge. Experiments to localize the main impulse generating area within the receptor are now underway.

The failure to record potentials from chemoreceptors in a large majority of the arthropods tested could result from a real absence of these receptors in the appendages tested or from limitations of the technique. The latter is the more probable explanation in most cases. Particularly unfortunate is the apparent inapplicability of the technique to recordings from the antennae of most insects. Unavoidable short circuits between indifferent and recording electrodes explain some negative results, as noted above, but inability to position the recording electrode over one or a few receptor sensilla and the small size of the spike potentials from the chemo-

receptors undoubtedly account for most of the failures. The optimum preparation for use with this technique appears to be an elongated sensillum, well isolated from surrounding sensilla, and containing very few receptor cells—an ideal approached more conveniently in the labellar chemoreceptors of flies than with any other arthropod preparations yet tested. A similar survey of the chemoreceptors of marine arthropods is planned.

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Summary

1. Electrophysiological tests with externally applied, fluid-filled electrodes were performed upon thirty-seven species representing four classes of arthropods. Afferent chemoreceptor impulses were recorded in animals of five types: a crayfish (*Cambarus*), a millipede (*Pseudopolydesmus*), two orthopterans (*Ceuthophilus* and *Hadenoecus*), a helomyzid fly (*Amoebaleria*), and six species of butterflies.

2. Receptors sensitive to chemical, tactile, and temperature stimuli within normal physiological ranges are found in certain Lepidoptera (*Epargyreus* and *Limenitis*) and Diptera (*Amoebaleria*). Receptors with a dual sensitivity to at least two of the above types of stimulation are found in *Pseudopolydesmus*, *Ceuthophilus*, and *Hadenoecus*. It is concluded that multiple sensitivities of receptors are not exceptional in arthropods.

3. Chemoreceptors sensitive to amino acids, but insensitive to tactile and temperature stimuli, are found on the chelae and protopodites of the first two walking legs of *Cambarus bartonii sciotensis*.

4. With the present recording method, spike potentials from chemoreceptors represent increases in positivity at the distal tip of the receptor cell, relative to the cell body.

5. Relationships between functional characteristics of chemoreceptors and the natural history of the animals are discussed.

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